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Prognostic Significance of bcl-2 Protein Expression in Aggressive Non-Hodgkin's Lymphoma

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Little is known about the expression of bcl-2 protein in intermediate and high grade non-Hodgkin's lymphoma (NHL) and its clinical and prognostic significance. We performed immunohistochemical analysis of bcl-2 expression in tumoral tissue sections of 348 patients with high or intermediate grade NHL. These patients were uniformly treated with adriamycin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) in the induction phase of the LNH87 protocol. Fifty eight cases were excluded due to inadequate staining. Of the 290 remaining patients, 131 (45%) disclosed homogeneous positivity (high bcl-2 expression) in virtually all tumor cells, whereas 65 (23%) were negative and 94 (32%) exhibited intermediate staining. High bcl-2 expression was more frequent in B-cell NHL (109 of 214, 51%) than in T-cell NHL (6 of 35, 17%) ($P = .0004$), and was heterogeneously distributed among the different histological subtypes. Further analysis was performed on the 151 patients with diffuse large B-cell lymphoma (centroblastic and immunoblastic) to assess the clinical significance and potential prognostic value of bcl-2 expression in the most frequent and homogeneous immunohistological subgroup. High bcl-2 expression, found in 44% of these patients (67 of 151), was more frequently associated with III-IV stage disease ($P = .002$). Reduced disease-free survival (DFS) ($P < .01$) and overall survival ($P < .05$) were demonstrated in the patients with high bcl-2 expression. Indeed, the 3-year estimates of DFS and overall survival were 60% and 61%, respectively (high bcl-2 expression) versus 82% and 78%, respectively (negative/intermediate bcl-2 expression). A multivariate regression analysis confirmed the independent effect of bcl-2 protein expression on DFS. Thus bcl-2 protein expression, as demonstrated in routinely paraffin-embedded tissue, appears to be predictive of poor DFS, in agreement with the role of bcl-2 in chemotherapy-induced apoptosis. It might be considered as a new independent biologic prognostic parameter, which, especially in diffuse large B-cell NHL, could aid in the identification of patient risk groups.

THE BCL-2 gene was initially discovered by virtue of its involvement in the t(14;18) (q32;q21) translocation.[1-3] This chromosomal abnormality, resulting in production of high levels of bcl-2 protein, is observed in the majority of follicular non-Hodgkin's lymphomas (NHLs) and in about 20% of diffuse large B-cell lymphomas.[4-7] However, the expression of bcl-2 protein is not restricted to B-cell lymphomas bearing the t(14;18) translocation.[8] Indeed, immunohistochemical studies have shown that, beside follicular lymphomas,[8,9] a broad spectrum of lymphoid malignancies including chronic lymphocytic leukemia, plasma cell dyscrasia, diffuse large B- and T-cell lymphomas,[10] as well as non-lymphoid tumors of lung,[11] breast,[12] prostate[13] or liver origin,[14] also express the bcl-2 protein. Finally, the bcl-2 protein is also detected in a number of normal tissues, including B lymphocytes of the mantle zone and normal T cells.[8,15]

Bcl-2 can be regarded as a member of a new category of oncogenes that is involved in cell survival, by blocking programmed cell death, also called apoptosis.[16] Deregulated bcl-2 extends the survival of some interleukin-dependent hematopoietic cell lines when deprived of growth factors.[17] In vivo, bcl-2-Ig transgenic mice express constitutively a high level of bcl-2 protein, and thus accumulate an excess of resting IgM/IgD B cells because of extended survival.[18] Thus, constitutive bcl-2 expression might cooperate with the activation of other oncogenes, such as c-*myc*, in a multistep development of lymphomas.[19]

It has been shown that apoptosis occurs in a majority of lymphoid cells when treated by various antineoplastic agents commonly used in the treatment of NHLs.[20,21] Recent in vitro studies on murine and human leukemia cell lines have demonstrated that, although not preventing suppression of cell proliferation, high levels of bcl-2 protein could protect the cells from undergoing apoptosis in the presence of glucocorticoids and multiple chemotherapic drugs.[22,23]

Taken together, these findings suggest that a high level of bcl-2 protein may play an important role both in lymphomagenesis and in the development of drug resistance, in follicular, as well as in other varieties of lymphomas. This would provide support for the potential clinical value of bcl-2 protein expression in NHLs, as recently suggested in acute myeloid leukemias[24] and lung carcinoma.[11] However, only few studies have focused on the correlation between bcl-2 expression at the protein level and clinical presentation and outcome in follicular[25] or diffuse[26,27] lymphomas. This prompted us to analyze the distribution of bcl-2 at the protein level according to histology and phenotype in 290 uniformly treated patients with intermediate or high-grade NHLs enrolled in the LNH87 protocol.[28] To assess its clinical significance and its potential prognostic value, we focused on the correlation between bcl-2 expression, clinical features, and outcome in diffuse large B-cell lymphomas, which is the most frequent and homogeneous immunohistological subgroup of the present series.

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MATERIALS AND METHODS

Patients studied for the expression of bcl-2 at the protein level are a subset of the 2,947 patients entered on the LNH87 protocol, a prospective multicentric trial of the Groupe d'Etude des Lymphomes de l'Adulte (GELA), between October 1, 1987 and April 1, 1992.[28-30]

LNH87 protocol design. Previously untreated adult patients with intermediate or high-grade NHL according to the Working Formulation (WF)[31] were stratified into three groups on the basis of age and prognostic factors and were then randomized to receive either ACVBP (adriamycin, cyclophosphamide, vindesine, bleomycin and prednisone), which was the reference induction treatment arm or another alternating anthracyclin-containing regimen. Eligibility criteria was as follows: patients under 70, with biopsy proven intermediate or high-grade histologies (WF, categories D through J). Exclusion criteria included prior treatment, a positive serology to the human immunodeficiency virus, concomitant or previous cancer (except in situ cervix carcinoma or skin epithelioma), heart disease, uncontrolled diabetes mellitus, liver or kidney failure. Patients with a previous history of low-grade NHL or with bone marrow or central nervous system involvement in the setting of Burkitt or lymphoblastic histology were also excluded. A centralized histological review and phenotypic study was strongly encouraged in the LNH87 protocol design. Thus, unstained slides of the initial biopsy specimen were available in 85% of cases. The histopathologic diagnoses were based on consensus review by three hematopathologists, and lymphomas were classified according to the WF and the Kiel classification.[32] The Ki-1 (CD30) positive anaplastic large cell subtype was added to the categories of the WF. Immunophenotypic studies were performed on deparaffinized tissue sections using a panel of monoclonal antibodies directed against B (CD20/L26, CDw75/LN1, MB2), and T (CD3, CD45Ro/UCHL1)-cell associated antigens to assess the B- or T-cell lineage of the lymphoma. Lymphomas were considered of B-cell derivation if tumor cells expressed CD20 antigen and/or were MB2 and CDw75 positive, but did not express CD3. They were considered of T-cell derivation if tumor cells expressed CD3 antigen or, if CD3 negative, were CD45Ro positive, and did not express CD20, CDw75 and MB2. Lymphomas were considered as having an undetermined phenotype when any other combination was observed.

Patient selection. We chose to analyze bcl-2 protein expression among patients uniformly treated by the ACVBP induction regimen. Among the patients with histologically reviewed NHL who received ACVBP, 348 were selected at random to analyze the distribution of bcl-2 expression according to histology and phenotype. Fifty eight cases were excluded due to inadequate tissue for bcl-2 staining. Histological and phenotypic characteristics of the 290 patients of the bcl-2 protein study group are indicated in Table 1. Among these 290 patients, 151 patients had diffuse large-cell (centroblastic and immunoblastic) lymphoma of B-cell origin, which represented the most important and homogeneous immunohistological subgroup. These were specially analyzed for clinical and prognostic correlations. Clinical features of these 151 patients and of the remaining 267 eligible patients (ie, with diffuse large B-cell NHL who received ACVBP) of the LNH87 protocol are compared in Table 2. The two groups did not differ for the distribution of either adverse prognostic factors or histological subgroups. Table 3 provides the distribution into the risk groups of the International Prognostic Index[33] for the 151 patients studied for bcl-2, as compared with the corresponding patients treated identically during the same period. These data indicate that the patient bcl-2 study group was representative of the patient population with diffuse large B-cell NHL entered on the LNH87 protocol. Thirteen patients in complete response were randomized to receive a high-dose chemotherapy regimen followed by autologous bone marrow transplantation; the remaining 98 good-responder patients were treated with the LNH 84 sequential chemotherapy.[34] No difference in disease-free survival (DFS) and survival between these two consolidative procedures has been demonstrated.[28]

Bcl-2 staining. Staining for bcl-2 was performed, as previously described[9] on deparaffinized sections by the alkaline-phosphatase/anti-alkaline-phosphatase (APAAP) procedure (Dako SA, Glostrup, Denmark), using a monoclonal antibody specific for bcl-2 (bcl2/124) (Dako SA). Optimum labeling was obtained by twice repeating the bridge and APAAP complex. All immunohistochemical analyses were performed in a single laboratory. One slide of each case was evaluated for bcl-2 staining by two investigators (O.H., P.G.). The 58 excluded patients were cases in which bcl-2 staining failed to show any positive reaction on both neoplastic cells and normal small lymphocytes that normally act as positive internal controls. Among the 290 remaining cases, three patterns of reactivity were distinguished according to the estimated percentage of malignant cells stained for bcl-2 (Fig 1). Cases with the most tumor cells positive (more than 60%) were considered as lymphomas with "high bcl-2 expression"; in these cases, tumoral cells were usually strongly labeled. Cases with heterogeneous labeling, ie, bcl-2 positive neoplastic cells admixed in the same area with bcl-2 negative neoplastic cells, were categorized as lymphomas with "intermediate bcl-2 expression". Cases with virtually all tumor cells negative (>95%), but with small reactive lymphocytes positive, were classified as "bcl-2-negative" lymphomas. For the analysis, areas of highest bcl-2 expression evident at lower power scanning were considered. The staining was considered as negative or intermediate after careful examination of the entire tissue section at high power scanning.

Statistical analysis. Patients' characteristics were compared using chi-square tests. The stopping date was fixed on March 31, 1994 providing a median follow-up of 44 months. The period of survival was calculated from the date of registration in the study to the stopping date or death or date of last follow-up when the stopping date was not reached. DFS was measured as the interval between the date of complete remission after induction treatment and stopping date, or relapse, death, or date of last follow-up evaluation when the stopping date was not reached. The rates of DFS and survival were estimated by the method of Kaplan and Meier[35] and were compared according to the treatment groups by log-rank tests.[36] Relative risks were estimated with the Cox regression model.[37] All significant tests were two-sided. Because the pattern "high bcl-2 expression" was unequivocal and easy to discriminate, we chose to compare the subgroup "high bcl-2 expression" with the other two.

RESULTS

Immunostaining for bcl-2 protein. Of the 290 patients studied, 131 (45%) disclosed high bcl-2 protein expression. In such cases, most, if not all, tumor cells were found to have bcl-2 protein. In addition, staining was usually strong with neoplastic cells more intensively stained than normal small lymphocytes. Among the remaining cases, 65 (23%) were considered bcl-2 negative, and 94 (32%) exhibited an intermediate staining with bcl-2 positive and bcl-2 negative neoplastic cells admixed in the same areas. The staining patterns are illustrated in Fig 1.

Bcl-2 expression and histological and immunophenotypic characteristics. The expression of bcl-2 according to histological subtypes and immunophenotype is shown in Table 4. Differences were noted according to bcl-2 expression among the different histological subgroups. All cases of diffuse small cleaved cell lymphoma (ie, mantle-cell NHL) exhibited high bcl-2 expression, whereas the latter pattern ranged from 32% to 58% of the other histological subgroups.

Table 1. Distribution of the 290 Patients Studied for bcl-2 Expression According to Histology and Phenotype

Histology		
WF	Kiel	N (%)
Diffuse small cleaved	Centrocytic,* 13	13 (4)
Diffuse mixed†	Centroblastic centrocytic, 5 Polymorphic immunocytoma, 10 Lymphoepithelioid (Lennert's), 6 Angioimmunoblastic (AILD)-type, 9	32 (11)
Follicular large cell	Follicular centroblastic, 24	24 (8)
Diffuse large cell (including immunoblastic)†	Centroblastic, 142 Immunoblastic B, 9 Pleomorphic T medium large, 10	183 (63)
Small noncleaved (Burkitt)	Burkitt, 5	5 (2)
Lymphoblastic	Lymphoblastic T, 5	5 (2)
Anaplastic‡	Anaplastic, 22	22 (8)
Unclassifiable	Unclassifiable, 6	6 (2)
Phenotype§		
B cell		214 (76)
T cell		35 (13)

Abbreviation: WF, Working Formulation.

* Mantle-cell lymphomas.

† 24 cases (2, diffuse mixed; 22, diffuse large cell) were of undetermined phenotype and were not subcategorized according to Kiel.

‡ The Ki1(CD30) anaplastic large cell subtype was added to the categories of the WF.

§ Not available in 8 cases; 33 (11%) cases were of "null" phenotype.

Of the 183 patients with diffuse large cell lymphoma (including immunoblastic lymphoma), 78 (43%) were shown to have high bcl-2 expression. This pattern was also found in 67 (44%) of the 151 patients with diffuse large cell lymphoma of B-cell origin.

With respect to phenotype, high bcl-2 expression in tumor cells was statistically more frequent in B-cell lymphomas (109 of 214, 51%) than in T-cell lymphomas (6 of 35, 17%) ($P = .0004$).

Bcl-2 expression and clinical features. Bcl-2 expression according to the clinical characteristics of the patients was studied in the whole group and in the group of patients having diffuse large B-cell lymphoma. For the group having diffuse large-cell lymphoma of B-cell phenotype, the "high bcl-2 expression" pattern was more frequent in patients with III-IV stage disease at presentation ($P = .002$) (Table 5) whereas, in the whole group, this pattern was more frequently associated with nodal presentation ($P = .03$), bone marrow involvement ($P = .04$), and III-IV stage disease ($P = .003$). In contrast, there was no association of bcl-2 expression with the other prognostic factors of the International Index[33]: lactate deshydrogenase (LDH), number of extranodal sites, and performance status.

Bcl-2 expression and outcome in diffuse large B-cell lymphomas. To evaluate the prognostic significance of bcl-2 protein expression, analysis of outcome was restricted to the patients with diffuse large B-cell lymphomas, because the latter represent the most frequent and homogeneous immunohistological subgroup in the present series. This demonstrated on the 151 patients with diffuse large B-cell lymphoma a significant difference in DFS ($P < .01$) and survival ($P < .05$) based on the level of bcl-2 expression (Table 5). Indeed, for patients with high bcl-2 expression, the 3-year estimates of DFS and survival were 60% and 61%, respectively, whereas the 3-year estimates of DFS and survival in the bcl-2 negative/heterogeneous subgroup were 82% and 78% as illustrated in Fig 2A and B. Multivariate analysis showed that only high bcl-2 expression ($P = .01$) and performance status ($P = .04$) adversely affected DFS. However, only performance status independently affected overall survival ($P = .002$).

DISCUSSION

In the present report, we have studied the expression of bcl-2 protein in an homogeneously treated group of patients with high-or intermediate-grade of NHL. We show that bcl-2 is expressed by lymphoma cells with a high frequency

Table 2. Clinical Features of the 151 Patients With Diffuse Large B-Cell Lymphoma Studied for bcl-2 Expression

Clinical Features	bcl-2 Study Group (n = 151) No. (%)	Remaining Eligible Patients (n = 267) No. (%)	Significant Difference
Age (>60 yr)	47 (31)	68 (26)	$P = .2$
Sex (male)	86 (57)	148 (55)	$P = .7$
Stage (III-IV)	88 (58)	146 (56)	$P = .7$
Performance Status (≥2)	30 (20)	66 (25)	$P = .3$
LDH (>1 N)	84 (58)	147 (58)	$P = .9$
Extranodal sites (>1)	28 (19)	38 (14)	$P = .2$
3-yr DFS (%)	72 ± 8	68 ± 7	$P = .3$
3-yr survival (%)	70 ± 8	65 ± 6	$P = .3$

These 151 patients are compared with the other eligible patients (with diffuse large B-cell NHL) entered on the LNH87 protocol in the same period.

Table 3. Distribution of Patients With Diffuse Large B-Cell Lymphoma in Risk Groups Using the International Prognostic Index

Score*	bcl-2 Study Group (n = 151) bcl-2 Positive	bcl-2 Study Group (n = 151) bcl-2 Negative/ Heterogeneous	(%)	Remaining Patients† (n = 267) (%)
0-1	45	18	(42)	127 (48)
2	20	23	(28)	70 (26)
3	13	20	(22)	40 (15)
4-5	5	7	(8)	30 (11)

BCL-2 study group is compared with other eligible patients entered on the LNH87 protocol in the same period.

* Score defined according to the international index: low, score 0-1; low-intermediate, 2; high-intermediate, 3; high, 4-5.

† There is no significant difference in risk group distribution between patients studied for BCL-2 expression and the other eligible patients ($P = .2$).

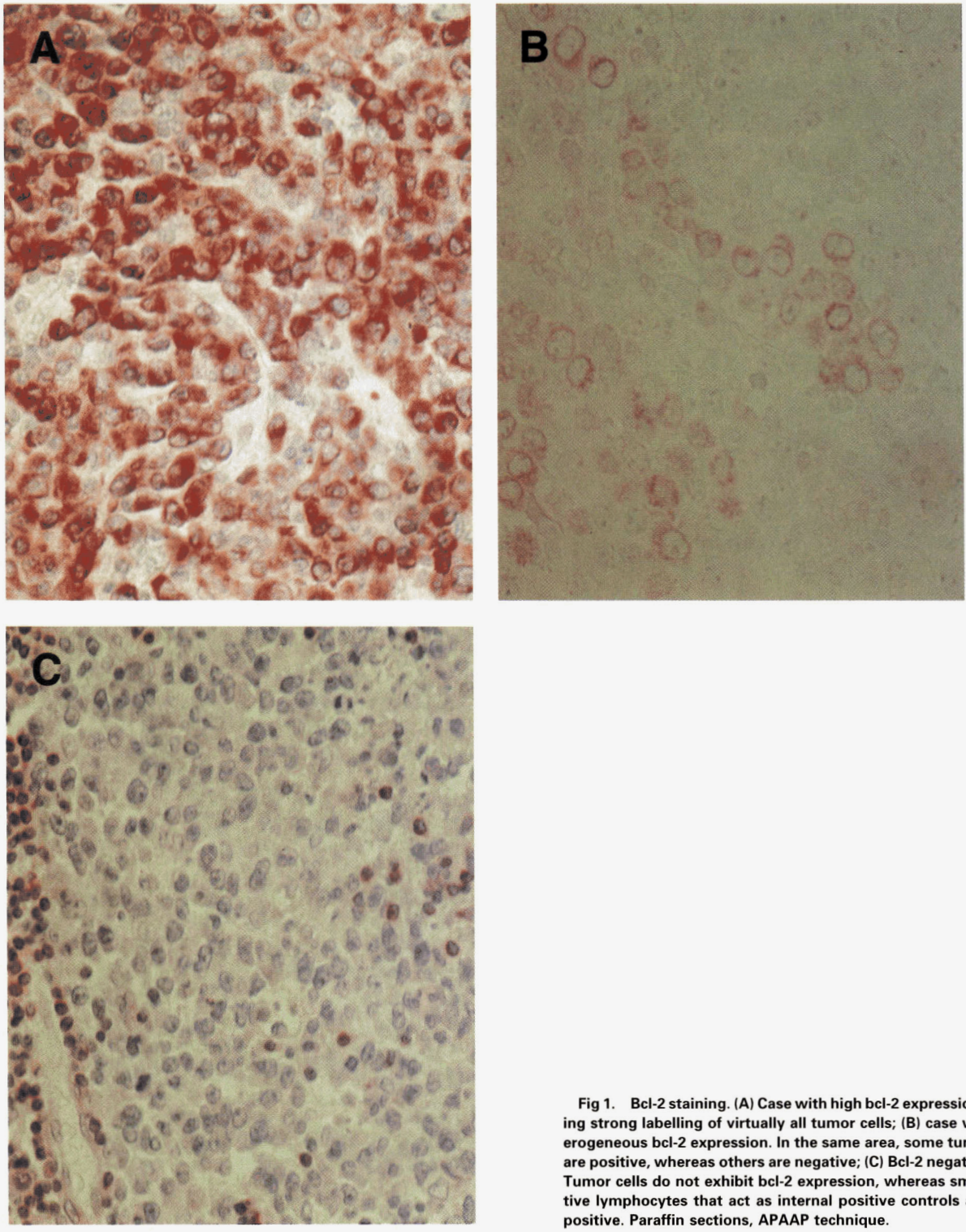

Fig 1. Bcl-2 staining. (A) Case with high bcl-2 expression showing strong labelling of virtually all tumor cells; (B) case with heterogeneous bcl-2 expression. In the same area, some tumor cells are positive, whereas others are negative; (C) Bcl-2 negative case. Tumor cells do not exhibit bcl-2 expression, whereas small reactive lymphocytes that act as internal positive controls are bcl-2 positive. Paraffin sections, APAAP technique.

regardless of histologic subgroups, and that high bcl-2 expression is correlated with B-cell phenotype. Furthermore, clinical correlations performed in the more homogeneous immunohistological group of diffuse large B-cell NHL disclose that high bcl-2 expression is correlated with extended stage of the disease and appears to be associated with a poor prognosis. Indeed, DFS and survival were significantly reduced in patients with high bcl-2 expression, although the independent effect of high bcl-2 expression, at the time of analysis, could be demonstrated only on DFS.

To date, most studies of the bcl-2 gene have focused on the presence of the t(14;18), which have been found in the

Table 4. Histological and Immunohistological Characteristics of the 290 Patients Studied for bcl-2 Protein Expression According to the Pattern of bcl-2 Staining

	bcl-2 Negative/ Heterogeneous	bcl-2 Positive
	No. (%)	No. (%)
Histology (WF, Kiel) No		
Diffuse small cleaved, 13	0 (0)	13 (100)
centrocytic,* 13	0 (0)	13 (100)
Diffuse mixed,† 32	20 (62)	12 (38)
Centroblastic centrocytic, 5	1 (20)	4 (80)
Polymorphic immunocytoma, 10	5 (50)	5 (50)
Lymphoepithelioid (Lennert's), 6	5 (83)	1 (17)
Angioimmunoblastic (AILD)-type, 9	9 (90)	1 (10)
Follicular large cell, 24	10 (42)	14 (58)
follicular centroblastic, 24	10 (42)	14 (58)
Diffuse large cell (includes immunoblastic),† 183	105 (58)	78 (43)
Centroblastic, 142	79 (56)	63 (44)
Immunoblastic B, 9	5 (56)	4 (44)
Pleomorphic T medium and large, 10	8 (80)	2 (20)
Small non-cleaved (Burkitt), 5	2 (40)	3 (60)
Burkitt, 5	2 (40)	3 (60)
Lymphoblastic, 5	3 (60)	2 (40)
Lymphoblastic (T), 5	3 (60)	2 (40)
Anaplastic,‡ 22	15 (68)	7 (32)
Anaplastic B, 9	2 (22)	7 (78)
Anaplastic T+nul, 13	13 (100)	0 (0)
Unclassifiable, 6	4 (66)	2 (33)
Total 290	159 (55)	131 (45)
Phenotype§		
B (n = 214)	105 (49)	109 (51)
T (n = 35)	29 (83)	6 (17)

* Mantle-cell lymphomas.

† 24 cases (2, diffuse mixed; 22, diffuse large cell) were of undetermined phenotype and were not subcategorized according to Kiel.

‡ The Ki1(CD30) anaplastic large cell subtype was added to the categories of the WF.

§ Not available in 8 cases; 33 (11%) cases were of "null" phenotype.

majority of follicular lymphomas, and in about 20% of diffuse large cell lymphomas.[4-7] It has been postulated, although not uncontroversially demonstrated, that the presence of a bcl-2 gene rearrangement or of the translocation t(14;18) seemed to be associated with shorter DFS and/or failure to achieve a complete remission.[7,26,38,39] In contrast, only a few studies have focused on the clinical relevance of bcl-2 expression at the protein level.[26,27] In the present study, we further extend to a large group of patients previous reports showing that the bcl-2/124 monoclonal antibody provides accurate staining even on routine paraffin-embedded biopsy material.[9,26] Using this antibody, we confirmed that high expression of bcl-2 is found in a large number (45%) of lymphomas with intermediate-or high-grade histology. The apparent discrepancy between the frequency of high expression of the protein and the bcl-2 gene rearrangement is consistent with the findings that bcl-2 protein is expressed in other hematologic malignancies such as acute[24] and chronic myelogeneous leukemia,[10] in a large variety of carcinomas from liver,[14] lung,[11] prostatic gland,[13] as well as in normal tissues, independently of the t(14;18) translocation.[8] These data strongly suggest that, beside the t(14;18) translocation, other pathological mechanisms may increase the expression of bcl-2. As an example, upregulation of bcl-2 expression has already been reported in vitro as a consequence of Epstein-Barr virus (EBV) infection[40] or interleukin-10 stimulation.[41] Alternatively, the transformation process may have occurred in subsets of normal B or T lymphocytes in which constitutive high levels of endogenous bcl-2 protein are present.[15]

This study further confirms previous observations that most high-grade mucosa-associated lymphoid tissue (MALT) lymphomas are bcl-2 negative,[42] as the latter pattern was shown in 12 of the 14 large B-cell lymphomas of the gastrointestinal tract (data not shown). The absence of detectable bcl-2 protein in a number of intermediate- and high-grade lymphoma, especially in high-grade MALT lymphomas, might reflect a breakdown in bcl-2 gene expression at the transcriptional or the posttranscriptional level.[43]

However, whatever the mechanism of deregulation of the bcl-2 gene, the finding of high expression of bcl-2 observed in a large proportion of NHLs raises the question of its clinical value and biological significance, because bcl-2 is now regarded as a member of a new category of oncogenes involved in blocking programmed cell death, thus leading to abnormal accumulation of cells.[16-18]

Our analysis, which was performed on fixed paraffin-embedded material, thus preserving morphology, easily identified three patterns of bcl-2 protein expression. We chose to individualize the group with virtually all tumor cells bcl-2 positive from the other two groups exhibiting either no or intermediate bcl-2 expression, because the labeling in the high bcl-2 expression group was homogeneous and easily distinguishable from the other two. In addition, several recent in vitro and in vivo studies have pointed out the physiological and clinical importance of high bcl-2 expression.[22,23,26,27] Furthermore, when comparing, in the present study, survival according to the level of bcl-2 expression, no difference was observed for the bcl-2 negative and bcl-2 intermediate groups. In the present series, including patients who were all treated with the same induction regimen of the LNH87 protocol containing high doses of anthracyclins and cyclophosphamide,[28] we show that the patients' outcome after chemotherapy was predicted by bcl-2 expres-

Table 5. Clinical Features of the 151 Studied Patients With Diffuse Large B-Cell Lymphomas According to bcl-2 Expression

	bcl-2 Positive (n = 64)	bcl-2 Negative/ Heterogeneous (n = 87)	
	No. (%)	No. (%)	*P* Value
Age (>60 yr)	25 (39)	22 (25)	.2
Sex (male)	36 (56)	50 (57)	.4
Stage III-IV	48 (72)	39 (47)	.002
Performance status (≥2)	16 (25)	13 (16)	.2
LDH > 1N	43 (65)	40 (51)	.1
Extranodal sites (>1)	12 (18)	16 (19)	.8
3-yr DFS (%)	60 ± 14	82 ± 10	.01
3-yr survival (%)	61 ± 12	78 ± 9	.05

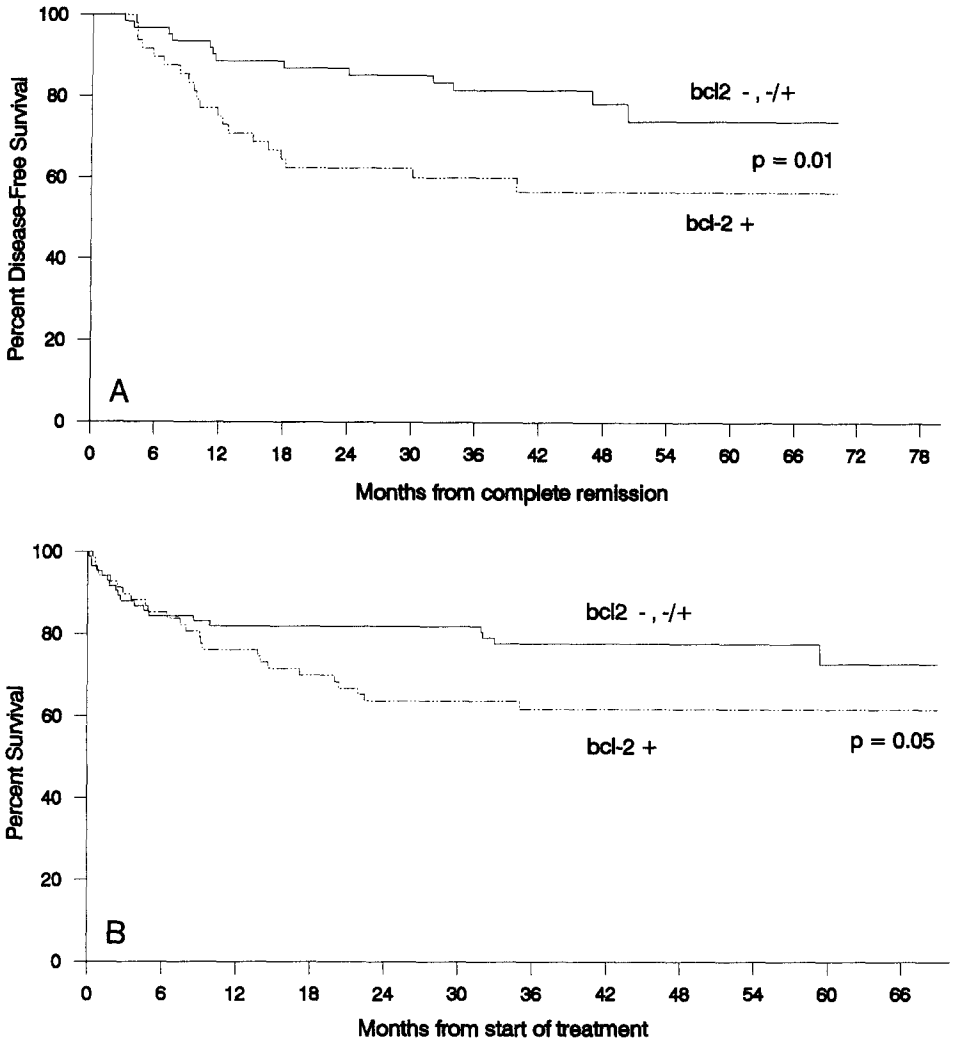

Fig 2. DFS (A) and overall survival (B) among patients with diffuse large B-cell lymphoma, according to status for bcl-2 protein.

sion. Indeed, for the whole group (data not shown), as well as for the more homogeneous subgroup of diffuse large B-cell lymphomas, relapses occurred more frequently and earlier in lymphomas of the high bcl-2 expression category. In addition, in the large B-cell NHL subgroup, survival was significantly reduced in patients with high bcl-2 expression. The prognostic differences between patients whose neoplasms show high bcl-2 expression and those that do not, may reflect different genetic and immunologic pathways for achieving similar histological tumors and clinical presentation rather than a direct effect of bcl-2. However, our finding is consistent with the recent in vitro studies of Myashita and Reed[22,23,44] who reported that bcl-2 transfected murine and human lymphoid cell lines exhibit higher resistance to several antineoplastic agents, including those used in the present study. Indeed, in bcl-2 transfected cells, chemotherapy induces arrest of proliferation, but unlike in control cells, death by apoptosis is prevented, and drug withdrawal results in reinitiation of cell growth.

In the more homogeneous subset of diffuse large B-cell lymphomas, high bcl-2 expression appears, in multivariate analysis, as an independent prognostic factor for DFS together with performance status. Therefore, it is not likely that the association of high bcl-2 expression with a poor clinical outcome is due to its correlation with stage III-IV disease. Moreover, the fact that high bcl-2 expression is not correlated with the level of LDH, suggests that the poor outcome is mainly due to delayed cell death or resistance to treatment, but not to increased cell proliferation. This hypothesis is consistent with the putative role of bcl-2 in lymphomagenesis, as a suppressor of apoptosis without effect on cell proliferation.[16] In contrast, the finding that high bcl-2 protein expression does not appear to affect independently overall survival of patients with diffuse large B-cell lymphomas may be due to an insufficient follow up, and thus should be reevaluated later.

In conclusion, in this series of uniformly treated patients with a diffuse large B-cell lymphoma, high bcl-2 protein expression was closely linked to reduced DFS and, at a lesser extent, to shorter survival. Together with the results of recent studies[26,27,45] performed on a smaller number of patients and/or receiving heterogeneous treatments, it appears that bcl-2 protein expression, as evaluated in routinely paraffin-embedded tissue, may be considered as a new independent biological

prognostic parameter. The bcl-2 protein expression, like the Ki67 proliferating index,[46] the presence of the lymphocyte homing receptor (CD44),[47] or deregulation of the p53 oncogene[27] may aid in the identification of patient risk groups.

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